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NITROGEN COMPOUNDS OF CABBAGE. I. THE RELATION OF THE NON-PROTEIN TO THE TOTAL NITROGEN WITH SPECIAL REFERENCE TO THE ESSENTIAL AMINO ACIDS

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In addition to its nutritive value, antibacterial, antifungal, antithyroidal and antiulcer properties have been ascribed to the fresh juice of cabbage. Although the antitbiotic, effective against Gram-positive and Gram-negative bacteria, has been described to be of a carbohydrate nature *(9)*, and the antifungal and goitrogenic substances to be polysulfides *(19, 20)*, none of these factors has as yet been clearly characterized. Moreover, there have been few comprehensive studies of any of the several classes of nitrogen compounds present in cabbage.

Perhaps the greatest concentration of interest centered on the nitrogen constituents of cabbage has revolved about the indole derivatives by researchers on natural plant growth (auxins) substances. Holley *et al (16)* first reported that the major indole was probably 3-indoleacetic acid. Neither Jones and Taylor *(21)*, nor Housley and Bentley *(17)* found any indication of the presence of this acid, although the latter workers admitted it may have been destroyed on extraction. Jones *et al (22)* isolated growth-promoting 3-indoleacetonitrile. While Denffer *et al (8)* and Fischer *(13)* claimed the presence of 3-indolealdehyde in cabbage based on paper chromatograms, it was Jones and Taylor *(21)* who recently isolated and identified this substance as well as 3-indolecarboxylic acid from both maturing and fully grown plants.

The thiocyanate and isothiocyanate content of cabbage has been investigated. The equivalent of 0.1% KCNS in the fresh tissue has been reported *(36)* while Gemeinhardt *(14)* found 302 γ % expressed as HCNS in freshly expressed juice. Jensen *et al (18)* found by paper chromatography that the allyl substituent is the predominant isothiocyanate in both white and red cabbage. In the former case this compound has been unequivocally confirmed *(28)*. The minor volatile compounds of this class differed. 3-Butenyl- *(18, 28)*, sec-butyl- *(28)* and probably sec-butenyl- *(18)* isothiocyanates were observed in two separate varieties of white cabbage while the benzyl derivatives were probably a constituent of the red variety *(18)*. Although uncharacterized polysulfides and a carbohydrate in cabbage have been credited with the antifungal and antibacterial properties, respectively, various isothiocyanates have been known for some time to exert inhibitory effects against these classes of organisms. The antithyroid compound 5-vinyl-2-thio-oxazolidone was isolated from cabbage seeds (1.5 g/kg) by Astwood *et al (3)* and

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recently, some evidence has been presented to indicate its possible presence in fresh cabbage leaf *(2)*.

Most of the known vitamins and vitamin-like substances in cabbage have been assayed. Table 1 summarizes the findings on the nitrogen-containing vitamins.

TABLE 1

Vitamin	Source	Quantity	Reference
		γ per gram fresh weight	
Thiamin	White cabbage	0.60–0.70	*(38, 42)*
	Red cabbage	1.00–1.50	*(38)*
Riboflavin	Cabbage	0.27	*(40)*
Pantothenic acid	Cabbage	1.2	*(40)*
Niacin	Cabbage	4–11	*(4)*
Folic acid	Cabbage	0.9 (free) 1.9 (total)	*(26)*
Folinic acid	Cabbage	0.18 (free) 0.41 (total)	*(26)*

Virtually no study has been published on the purine and pyrimidine nitrogen content of cabbage. deMan and deHeus *(31)* had found the purine nitrogen to equal 4.55% of the total nitrogen.

Although few complete amino acid and protein investigations have been undertaken, the isolation and characterization of two new sulfur amino acids from the non-protein nitrogen fraction of cabbage leaf has proven to be of considerable interest. McRorie *et al (32)* isolated and identified 3-amino-3-carboxypropyldimethylsulfonium salt, an analogue of methionine. This compound has been shown to be a source of labile methyl groups in transmethylation reactions and is able to substitute for methionine in a variety of organisms and enzymic systems. It apparently is not identical with the antiulcer factor reported in cabbage *(1)*. Although S-methyl-L-cysteine sulfoxide isolated from cabbage by Synge and Wood *(43)* is not a compound whose metabolic significance in the plant is easily understood, it represents a very substantial proportion of the organic sulfur of cabbage *(34, 43)*. Moreover, it has been shown that dimethyl sulfide and H_2S, the main substances accounting for the disagreeable odor of cooked cabbage, arise from this newly discovered sulfoxide *(6, 15)*.

In some of the early recorded work, small quantities of choline and betaine has been isolated from cabbage *(45)*. Rau and Ranganathan *(37)* had made a study of various nitrogen fractions of the non-protein nitrogen. Some of their findings would probably now require re-examination in the light of development of improved analytical methods. Such re-examination of earlier findings applies to the work of Schryver *et al (39)* who had reported hydroxylysine in cabbage leaf protein (1.55% hydroxylysine N of the total leaf albumin nitrogen). In other work of this period, Davies *(7)* had extracted and studied the protoplasmic proteins of cabbage leaf by the chemical methods afforded at the time. Employing microbiological methods Lyman and Kuiken *(29)* and Edwards, *et al (12)* have assayed the essential amino acids in whole cabbage. Very recently, Majunder *et al (30)* have reported on the amino acid composition of cabbage employing quantitative paper chromatography. In all of this work, however, no effort was made to

distinguish between the protein and non-protein fractions. In the analyses by earlier workers *(37, 39)*, the presence of hydroxyproline was suggested; presently, Majumder *et al (30)* also indicate the existence in cabbage of this uncommon amino acid. Some work on the enzymes of cabbage has shown the presence of peptidase activities toward leucyl and glycyl peptides *(5, 10)* but the identity of those peptides actually present in cabbage has not been disclosed.

The reported isolation of the alkaloid narcotine from cabbage *(27)* probably represents a most unusual find. However, up to the present time no published work has corroborated the presence of this compound in cabbage.

Cabbage is an important food item in the American diet. Nearly a million and a half tons are produced yearly for food, of which about a quarter of a million tons are processed, mainly as canned sauerkraut. The million and a quarter tons grown for fresh market are used for cooked cabbage, coleslaw, pepper hash, salad mixes and uncooked sauerkraut.

The objective of the present work was to study the distribution of total nitrogen and Van Slyke amino nitrogen in the protein and non-protein fractions of cabbage and to determine the amounts of the "ten" essential amino acids in whole cabbage and in both fractions after separation by electrodialysis and other methods.

This basic information is necessary to the determination of the role of the various nitrogen compounds in the deteriorative changes which occur during processing and storage of vegetable products.

EXPERIMENTAL PROCEDURES

Preparation of material. Wisconsin and Danish strains of Copenhagen Market cabbage were harvested from nearby fields in lower Bucks County, Pennsylvania. They were harvested in separate years during different periods of the year at market maturity by selecting, at random, 24 heads of uniform size and shape from large fields of this vegetable.

The coarse outer leaves were removed and the heads were thoroughly rinsed to remove any spray residue or other foreign matter. Each head was quartered and most of the core was removed in the form of a wedge after which the leafy quarters were coarse chopped and the chopped material from all 24 heads was mixed. A portion of this mixture was removed for alcohol extraction and the remainder frozen and stored at −20°C. When needed for further experimental determinations, the frozen cabbage was ground with dry ice to about 2 mm in a cold Wiley Mill and the dry ice allowed to evaporate from the frozen material. For electrodialysis studies a slurry made from this material with distilled water was circulated several times through a cold colloid mill at 0.025 mm between plates.

Separation of non-protein from protein fraction. Electrodialysis was the principal method used for the separation of the non-proteins from the proteins of cabbage. Since this procedure often produces heating in its initial stages, it was necessary to provide adequate cooling and stirring plus voltage control to prevent this heat formation at any point in the electrodialysis cell. Figure 1 shows the cell used in these experiments. It consists of three 8½ x 8½ x 2 in lucite blocks hollowed to make 3 chambers, each holding approximately 1 liter of liquid when clamped together with parchment paper sheets as dialysis membranes. Each chamber contains a U-shaped cooling coil of thin-walled ⅜ in. diameter tin tubing through which an aqueous alcohol coolant can be circulated from a refrigerated cooling bath. The center chamber into which the cabbage slurry was placed was stirred by means of a standard laboratory stirrer equipped with a glass paddle. The cathode and anode chambers containing 2 in square platinum electrodes were stirred by employing rapidly rotating teflon coated magnetic stirring bars held in place against the polished lucite faces of the outer chambers by magnetic stirring motors. All 3 chambers were fed through stoppered holes in the tops and drained

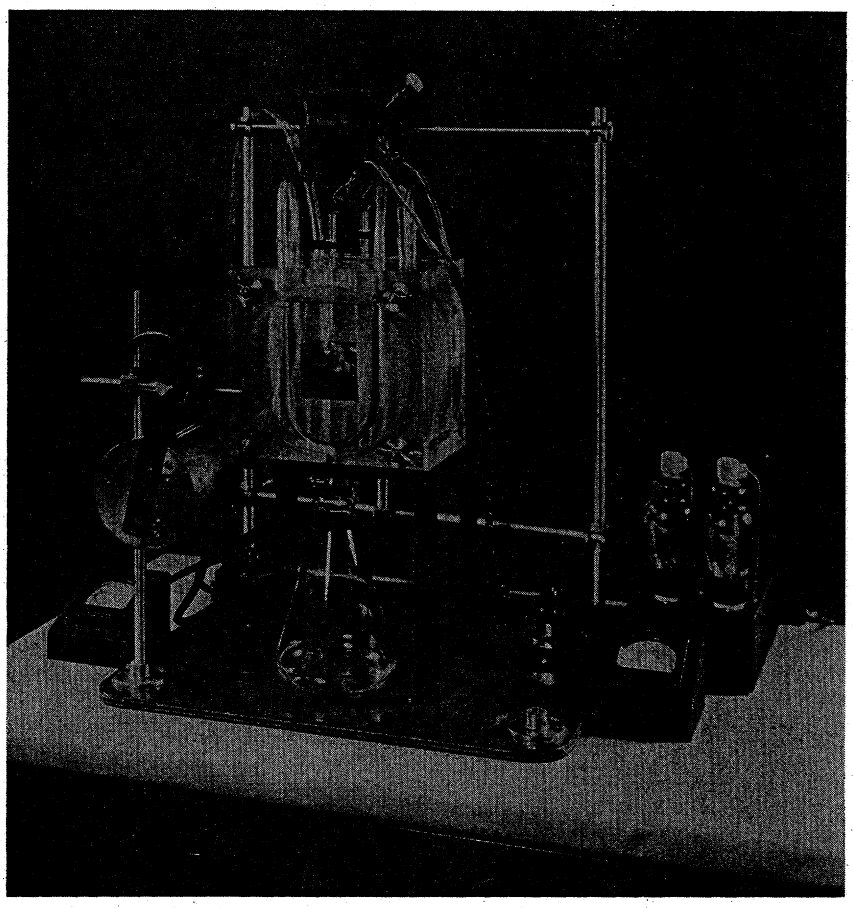

Figure 1.

through stainless steel petcocks in the bottoms of the chambers. Continuous distilled water flow could be maintained in this electrodialysis cell but in the experiments described in this paper the operation was carried out batchwise. DC voltage from 0 to a maximum of 250 v at 500 ma was provided by a "Variac"[b] controlled power pack. The temperature of the center chamber was maintained at 3–7° C by rapid circulation of the coolant and careful application of the current during the initial stages of an experimental run. Dialysis was considered complete when the current remained below 10 ma at 250 v DC for several hours.

Approximately 15 g MFB (moisture free basis) of colloidized cabbage suspension was placed in the center compartment and dialyzed against distilled water in the anode and cathode chambers. pH values were obtained at frequent intervals. The outer cell solutions were replaced with distilled water. Electrodialysis was continued until no appreciable current flowed through the cell (5 or 6 changes). Cathode solutions were acidified with HCl after removal from the cells and stored at 3° C until all the portions were collected. The respective dialysates were combined and concentrated to a volume of approximately 150 ml by vacuum concentration at 30° C, then made to a known volume for analysis. The residue fraction was washed out of the center cell with cold distilled water and made up to 2 liters. Aliquots were removed for analysis and the remainder was dried by freeze drying.

In an alternate procedure non-protein nitrogen was also extracted with 70% aqueous ethanol by the method described in the second paper of this series *(46)*.

A supplementary method of extraction was carried out by refluxing about 15 g (MFB) of colloidized cabbage in 300 cc of water for 2 hr, followed by filtration with subsequent washing of the residue with hot water. The filtrate was concentrated as before and analyzed for Kjeldahl nitrogen, arginine, threonine, and lysine. An aliquot of the hot water extract was made to 70% alcohol concentration with absolute ethanol and the slight amount of precipitate formed was removed. Both fractions were analyzed for total nitrogen and the 3 amino acids mentioned above.

Methods of analysis. Solids determinations on all samples were made by drying the samples to constant weight in a vacuum oven at 70° C. The Kjeldahl nitrogen were determined by the official A.O.A.C. method *(33)* using semi-micro Kjeldahl apparatus. Amino-nitrogen determinations were made by the Van Slyke method with a modified auxiliary reaction chamber as used by Doherty and Ogg *(11)*. Nitrate nitrogen was determined by the method of Jones and Underdown *(23)*.

Ten amino acids—arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, valine, threonine and tryptophan—were determined quantitatively by the microbiological method of Stokes *et al*, as modified by Kelley and Baum *(24)*. Aliquots of all fractions, either vacuum concentrated or lyophilized, were hydrolyzed in 10 cc of 10% HCl in sealed ampules for 10 hr in an autoclave at 15 lb pressure. A 2 hr hydrolysis was used for the analysis of methionine *(24)*. Alkaline hydrolysis was used for the analysis of tryptophan. Unhydrolyzed samples of the electrodialyzed cathode fractions gave unsatisfactory lysine values when the organism *Streptococcus faecalis* R was used. When *Leuconostoc mesenteroides* P 60 was tried using Difco Bacto Lysine Assay Medium,[b] consistently satisfactory results were obtained on these fractions. Electrometric titration of the lactic acid formed in this microbiological procedure is now being used in place of the colorimetric titration used in the above method *(24)*.

RESULTS AND DISCUSSION

Table 2 shows the distribution of solids, Kjeldahl nitrogen and Van Slyke amino nitrogen in different fractions of 2 strains of Copenhagen Market cabbage. In view of the nature of the raw materials the results show a remarkable area of agreement in the distribution of both solids and nitrogen in the 2 batches of cabbage used in these experiments.

It is of interest to note the high percentage of solids and nitrogen in the soluble fractions, and the close agreement between the amounts obtained by dialysis and by 70% alcohol extraction. The per cent of Van Slyke amino nitrogen follows a similar pattern. Distribution of amino N in the cathode, anode and residue fractions is that obtained under the conditions of uncontrolled pH changes which occur during the course of the dialysis. These changes will be noted later in the discussion. The recovery of amino N in the 3 fractions amounts to approximately 96% of the Van Slyke amino N of the whole cabbage. The low value of 2.4% amino N in the residue after dialysis indicates that the nitrogen of the residue (47% of the total N) is principally in the form of undialyzable polypeptitde and protein N. The present paper deals primarily with the soluble N compounds, but preliminary work indicates the protein is largely in the solid phase of the residue in the central compartment since the supernatant solution, although ninhydrin positive, gives no protein precipitate. This work will be reported in a subsequent paper on cabbage nitrogen components.

The nitrate nitrogen of cabbage is not listed with the total N in Table 2 since it is less than 0.1% as determined by the method of Jones and Underdown *(23)*.

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[b] Mention of this name does not imply endorsement of this product by the Department of Agriculture over any other similar products not named.

TABLE 2

Distribution of nitrogen and solids in cabbage fractions

Fraction	Strain	Solids		Total N mg/g (MFB)	% of total N	Van Slyke amino N mg/g (MFB)	Van Slyke amino N as % total N	Van Slyke amino N as % soluble N
		mg/g Fresh wt.	% of total solids					
Whole Cabbage	1[1]	73.6		25.4		7.75	30.5	
	2[2]	56.3		29.6		10.54	35.6	
Soluble								
Dialysate	1	48.9	66.5	14.0	55.1	6.43	25.3	45.9
	2	38.5	68.3	17.0	57.3	9.31	31.45	54.9
Alcohol extract	1	49.2	66.9	14.4	56.6	6.32	24.9	44.0
	2	40.0	71.0	17.3	58.4	10.03	33.9	59.2
Dialysate								
Cathode	1	24.5	33.3	9.35	36.8	5.26	20.7	48.1
	2	21.8	38.8	10.39	35.1	7.34	24.8	57.1
Anode	1	18.3	24.8	4.65	18.3	1.52	6.0	
	2	16.7	29.6	5.92	20.0	1.97	6.65	
Non-Dialyzable Residue	1	27.8	37.7	12.09	47.7	0.61	2.4	
	2	17.2	30.6	13.62	46.0	0.89	3.0	

[1] Strain 1 refers to Wisconsin Copenhagen cabbage.

[2] Strain 2 refers to Danish Copenhagen cabbage.

Table 3 gives the values for the 10 amino acids commonly referred to as the essential amino acids in hydrolyzed whole cabbage and in the fractions obtained by electrodialysis of a cabbage slurry. Both anode and cathode fractions were studied but all 10 amino acids listed in this table are found only in the cathode fraction under the conditions of our dialysis experiments. The table shows that most of these amino acids are quantitatively accounted for in the cathode and residue fractions as evidenced by the recovery figures in the last 2 columns. Of the 10 "essential" amino acids in cabbage, arginine is the only one present in greater amounts in the non-protein than in the protein fraction (68.8% in the cathode to 36.5% in the residue). The other 9 acids in the cathode range downward from 36.3% for histidine to 2.2% for tryptophan.

The cathode fractions were analyzed both before and after hydrolysis with 10% HCl and the differences can be noted in Table 3.

It is difficult to explain these differences particularly in the case of arginine where the amount of this amino acid in the cathode is considerable. With 83.4% of the arginine in whole cabbage recovered in the unhydrolyzed cathode compared to 68.8% in the hydrolyzed fraction it is indicated that some growth stimulant is present in the unhydrolyzed cathode. It is possible that short chain peptides passed through the membranes and acted as a stimulant to the growth of the test organism in the manner described by Klungsøyr *et al (25)*. One would expect these peptides to be split by the 10 hr autoclave hydrolysis and this appeared to be the case with the Wisconsin strain of cabbage since the total recovery of arginine in the residue and the hydrolyzed cathode was 105% as compared to the 120% recovery for the residue plus the unhydrolyzed cathode.

Recovery of lysine in the cathode fractions was more variable in the different runs than any of the other amino acids, particularly in the unhydrolyzed cathodes. When *Streptococcus faecalis* R was used as the assay organism the lysine recovery from the unhydrolyzed samples and the residue was as low as 80% of the total lysine indicated by the value in the whole cabbage. Somewhat better recovery was obtained when *Leuconostoc mesenteroides* P60 was used (85–95%).

Tryptophan was apparently partially destroyed by the dialysis treatment and the amount in the cathode was so small that accurate analysis was difficult.

Table 4 has been designed to show the total and Van Slyke amino nitrogen distribution of each of the ten amino acids in the Wisconsin strain of Copenhagen Market cabbage calculated from the values in Table 3. Table 4 also shows the relationship of each and the sum of the 10 acids to the total N and Van Slyke amino N as found for whole cabbage and for the cathode fraction.

With an average of 97.5% recovery of the essential amino acids in the cathode and residue fractions it is interesting that these acids account for 32.6% of the total N in the whole cabbage with 11.9% in the cathode after electrodialysis. 36.6% of the cathode N is due to the N of the essential amino acids. As shown in Table 2, 30.5% of the nitrogen in cabbage was determined as Van Slyke amino N and 62.3% of this N was accounted for by the 10 essential amino acids in the whole cabbage and 15.25% in the cathode. The Van Slyke amino N in the cathode essential amino acids is 4.65% of the total

TABLE 3

Microbiological assay of 10 amino acids in cathode and center cell residue of electrodialyzate and whole cabbage (Wisconsin Copenhagen)[1]

Amino acid	Whole cabbage mg/g (MFB)	Cathode				Residue hydrolyzed		Recovery %	
		Unhydrolyzed		Hydrolyzed					
		mg/g (MFB)	% of total amino acid	mg/g (MFB)	% of total amino acid	mg/g (MFB)	% of total amino acid	Cathode unhydrolyzed + residue	Cathode hydrolyzed + residue
Valine	5.7	0.9	15.8	1.1	19.3	4.7	82.5	98.3	101.8
Histidine	2.2	0.9	40.9	0.8	36.3	1.2	54.5	95.4	91.0
Arginine	9.6	8.0	83.4	6.6	68.8	3.5	36.5	119.9	105.3
Leucine	6.7	0.5	7.5	0.5	7.5	5.7	85.1	92.6	92.6
Threonine	5.1	1.3	25.5	1.2	23.5	3.7	72.5	98.0	96.0
Methionine	1.9	0.1	5.3	0.2	10.5	1.7	89.5	94.8	100.0
Lysine	7.5	0.9	12.0	1.1	14.7	5.5	73.4	85.4	88.1
Tryptophan	0.9	0.04	4.5	0.02	2.2	0.5	55.5	60.0	57.7
Isoleucine	4.6	0.5	10.9	0.9	19.6	3.9	84.8	95.7	104.4
Phenylalanine	4.9	0.51	10.4	0.38	7.8	4.2	85.7	96.1	93.5

[1] The figures in this table are the average of 2–5 replicates of at least three dialysis experiments.

TABLE 4

Distribution of total and amino nitrogen calculated for the 10 essential amino acids in whole and soluble cabbage fractions

Amino acid	Total N of amino acid as % of total nitrogen		Total N of amino acid as % of total soluble [1] nitrogen	Amino N of amino acid as % of total nitrogen		Amino N of amino acid as % of total amino nitrogen	
	Whole cabbage	Cathode fraction	Cathode fraction	Whole cabbage	Cathode fraction	Whole cabbage	Cathode fraction
Valine	2.68	0.52	0.94	2.69	0.52	8.80	1.70
Histidine	2.14	0.85	1.55	0.78	0.28	2.55	0.93
Arginine	12.12	8.37	15.20	3.04	2.09	9.95	6.85
Leucine	2.81	0.21	0.38	2.81	0.21	9.23	0.68
Threonine	2.36	0.55	1.01	2.36	0.56	7.74	1.82
Methionine	0.70	0.07	0.14	0.70	0.07	2.30	0.25
Lysine	5.67	0.83	1.51	5.67[2]	0.83	9.25	1.35
Tryptophan	0.49	0.01	0.02	0.24	0.003	0.80	0.01
Isoleucine	1.93	0.38	0.68	1.93	0.38	6.34	1.24
Phenylalanine	1.64	0.12	0.23	1.64	0.13	5.37	0.41
Total of 10 essential	32.60	11.91	21.66	21.89	5.06	62.32	15.25

[1] Cathode and anode.

[2] Lysine yields from 80–90% of its ϵ amino N by the Van Slyke method.

N. As noted in Table 4 the Van Slyke amino N of lysine is greater than its theoretical value based on α-amino N only.

Data in Table 5 were obtained in order to compare the recovery of arginine, threonine and lysine using electrodialysis, aqueous ethanol and hot water (100° C) extractions, and to determine the values for these 3 amino acids in the Danish strain of Copenhagen Market cabbage. Our interest in the use of aqueous ethanol and hot water as extractants of the soluble nitrogen constituents of vegetables came about as a result of our chromatographic studies *(46)* in which aqueous alcohol was used as the extractant. Arginine and lysine values were lower in the alcohol extracts analyzed by the chromatographic method than in the electrodialyzed cathode fraction analyzed by the microbiological procedure. Oland and Yemm *(35)* reported difficulty in quantitatively extracting arginine from apple stem tissue with 70% aqueous alcohol and they removed about 20% more arginine by hot water extraction.

TABLE 5

Total nitrogen and amino acids in Danish Copenhagen cabbage fractions as determined on hydrolyzed extracts by microbiological assay

Sample	Kjeldahl N % (MFB)	Arginine		Threonine		Lysine	
		mg/g (MFB)	% of total arginine	mg/g (MFB)	% of total threonine	mg/g (MFB)	% of total lysine
Whole cabbage slurry	2.95	9.2		5.0		6.4	
Cathode	1.26	6.7	72.8	1.3	26.0	1.4	21.9
Anode	0.43	—	—	—	—	—	—
Residue	1.22	4.0	43.5	4.0	80.0	5.0	78.1
Recovery %	98.6		116.3		106.0		100.0
EtOH extract	1.72	6.2	67.4	1.6	32.0	0.9	14.1
Residue	1.17	4.2	45.7	3.6	72.0	5.0	78.1
Recovery %	98.0		113.1		104.0		92.2
Hot H_2O extract	1.88	6.3	68.5	1.7	34.0	1.7	26.6
Residue	0.94	3.4	39.1	2.8	56.0	4.0	62.5
Recovery %	97.3		107.6		90.0		89.1

In Table 5 all extractions were made simultaneously from identical batches of cabbage and all samples were hydrolyzed and analyzed for arginine, threonine and lysine by the microbiological method. Threonine was included in this group because previous values were in good agreement by both methods of extraction and analysis.

Total nitrogen values are included in this table for better comparison of the three extracts. It can be seen that the combined cathode and anode fractions containing 1.69% of the total N of whole cabbage and the ethanol extract containing 1.71% N are in good agreement but the hot water extract with a value of 1.88% N is about 10% higher in total N. The hot water residue, on the other hand, is about 13% lower than the average of the dialysis and alcohol residues. Total recovery of nitrogen in each extract is close to 98%.

Arginine values in the 3 extracts are in good agrement and the cathode value for the Danish strain is very close to the averaged value found for this amino acid in the Wisconsin strain.

Extraction of arginine by aqueous alcohol was apparently complete since neither dialysis nor hot water removed a significantly greater amount from this cabbage tissue.

Arginine in the residue from the Danish cabbage was higher than that found in the residue from the Wisconsin strain and again the total recovery was high (116%) of the whole cabbage. Since both the cathodes and residues and the ethanol extracts were hydrolyzed in these experiments, it is difficult to understand how a peptide could account for a more rapid growth rate of the test organism. Further study will be needed to determine the actual existence in cabbage of such a growth factor and what its nature might be.

Data in Table 5 show that both alcohol and hot water appear to extract a somewhat larger amount of threonine than electrodialysis but the total recovery of this amino acid in all 3 extracts is within the ± 10% limit of this method of assay. Likewise aqueous alcohol appears to extract less lysine than either dialysis or hot water, but at the low levels at which this amino acid occurs in the soluble fractions the differences may not be significant. Recoveries of both threonine and lysine in the residue fractions of the hot water extraction experiments are appreciably lower than those in the dialysis and alcohol extraction, but since total recoveries are close to 90% even more accurate methods of analysis will be needed to explain these differences.

In this paper we have attempted to show the relationship of the free essential amino acids to the protein-bound ones and to determine whether certain methods of extraction are more suitable than others for the separation of free and bound amino acids. In the case of the amino acids studied so far, electrodialysis, aqueous alcohol, and hot water extraction methods are equally suitable. This same statement cannot be made for certain other amino compounds present in cabbage tissue in considerable amounts. Zacharius *et al,* in the second paper of this series *(46),* have identified and estimated an additional 11 amino compounds and postulated the presence of others which contribute to the total nitrogen of the tissue extract. A complete nitrogen balance can be obtained only after all of these compounds are quantitatively determined.

In the electrodialysis method of separation of non-protein from protein amino compounds at least 2 substances greatly complicate the study of the nitrogen distribution in vegetable tissue. These are the amides glutamine and asparagine. Table 2 shows that all of the nitrogen can be recovered in the anode, cathode and residue fractions but the distribution is not always the same from run to run. This was shown particularly by the Danish strain in 2 different electrolytic separations. Since the recovery of 10 of the amino acids is consistent, it is probable that changes in the nitrogen distribution in the three compartments are the result of the breakdown of the amides. In particular, glutamine can be converted to pyrrolidone carboxylic acid or glutamic acid with the concurrent release of ammonia. Both acids would be expected to migrate to the anode compartment.

The pH changes that occur during electrodialysis are probably a result of the breakdown of this compound and of asparagine *(46).* Starting with an initial pH of 5.8 in the cabbage slurry the pH of the cathode reached 10.4 within 15 min and 12.0 within 1½ hr. It dropped to pH 7.5 after 3 hr. The central cell dropped to pH 3.0 in 15 min and rose to pH 3.4 in 3 hr remaining

close to this figure throughout a run. The anode dropped as low as pH 2.0 in 15 min and rose to pH 2.7 in 3 hr. At no time could any ammonia odor be detected and it is probable that ammonium salts were formed in the dialysis cells; these could account for the pH drop in the cathode.

At present it seems possible to conclude that electrodialytic separation of the essential amino acids and possible others is practical but that alcohol extraction is better for the extraction of all the free amino compounds of cabbage tissue. For separation of protein and non-protein nitrogen fractions, electrodialysis methods should be more suitable since no coagulation of the protein compounds can occur at the low temperatures maintained under our experimental conditions.

Since no comparable data on free amino compounds in cabbage can be found in the literature it is impossible to compare our values with those of others. Majumder *et al (30)* found no measurable arginine or methionine in certain extracts which they made, but it is difficult to deny the presence of these amino acids in the free form in all of our extracts. Total values for the essential amino acids in whole cabbage have been found by Lyman and Kuiken *(29)* and Edwards *et al (12)* and our values for these same acids are in good agreement with their findings.

SUMMARY

Non-protein nitrogen compounds of two strains of Copenhagen Market cabbage have been separated from the protein constituents by electrodialysis, 70% aqueous alcohol extraction and hot water extraction. Whole cabbage, extracts and residues have been analyzed for total solids, total nitrogen and α-amino nitrogen (Van Slyke N). Ten "essential" amino acids have been quantitatively determined by microbiological methods in whole cabbage and in cathode fractions after electrodialysis. Arginine, threonine and lysine have been compared in whole cabbage, electrodialyzed, alcohol, and hot water extracts. The total nitrogen and Van Slyke N contents of these acids have been calculated and compared with the total N and Van Slyke N of whole cabbage and its separated fractions.

Total solids and total nitrogen values of the two strains of cabbage differed by as much as 30% and 16%, respectively, but the extractable solids and total nitrogen differed by only 2%.

Total solids and nitrogen for the combined dialysate (cathode and anode) and the 70% alcohol extract for each strain were in close agreement. The Van Slyke N of the Danish Copenhagen Market strain was about 6% higher in the dialysate and 9% higher in the alcohol extract than the Wisconsin strain. This was estimated to be due largely to the amide glutamine as determined by chemical methods described in a second paper of this series.

Hot water extracted a higher percentage of total N than either of the other methods. Total solids, total N and Van Slyke N in the various extracts and the residues from these extracts gave from 97 to 100% recovery based on the values found in the whole cabbage.

Distribution of total nitrogen in the cathode and anode fractions of the dialysate differed by as much as 10% in different runs; this was thought to be largely due to the destruction of the amide glutamine which was broken down by electrodialysis but not by alcohol extraction.

The amino acids arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine were recovered quantitatively in cathode and residue fractions after electrodialysis (±10%). Lysine recovery was somewhat variable in different sample runs and a little on the low side. Arginine recovery was about 20% high when the cathode was not hydrolyzed before analysis and the recovery from the Danish strain was a little higher than that from the Wisconsin strain. Tryptophan was partially destroyed by electrodialysis and yielded only 60% of that found in hydrolyzed whole cabbage.

Arginine was the only "essential" amino acid present in larger amounts in the soluble fraction that in the protein fraction.

Electrodialysis and alcohol extraction methods were equally suitable for separation of nine of the essential amino acids but alcohol extraction is preferred if other amino compounds are desired. Electrodialysis would be preferred as a method of separation of non-protein amino compounds from protein if the protein were to be used for further study.

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